201-14327

**NCIC HPV**
Sent by: Mary-Beth Weaver

03/05/2003 01:20 PM

To: NCIC HPV, Jodi Burgess/DC/USEPA/US@EPA
cc:
cc:
Subject: Public Comments on the HPV Test Plan for Estragole

**Chad Sandusky <csandusky@pcrm.org> on 03/04/2003 01:38:33 PM**

Please respond to csandusky@pcrm.org

To: oppt.ncic@epamail.epa.gov, hpv.chemrtk@epamail.epa.gov, Rtk Chem/DC/USEPA/US@EPA, Karen Boswell/DC/USEPA/US@EPA, tadams@therobertsgroup.net

cc:

Subject: Public Comments on the HPV Test Plan for Estragole

Attached please find the comments and attachment to same of the American animal protection community on the High Production Flavor and Fragrance High Production Volume Consortia (FFHPVC) HPV test plan for estragole.

We look forward to your comments.

Regards,

Chad B. Sandusky, Ph.D.
Senior Toxicologist
Physicians Committee for Responsible Medicine
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202-686-2210 ext. 302

Estragole-HPV-3-3.pdf Estragole-HPV-attachment.pdf

201-14327

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March 4, 2003

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Comments on the HPV Test Plan for Estragole

Dear Administrator Whitman:

The following comments on the High Production Flavor and Fragrance High Production Volume Consortia (FFHPVC) HPV Challenge test plan for estragole are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

FFHPVC submitted its test plan on October 21, 2002. Estragole (CAS No. 140-67-0) is currently permitted by the FDA for direct addition to food for human consumption as a flavoring substance and is considered by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavoring substance. As stated in the test plan, estragole occurs naturally in more than 39 foods and exposure occurs primarily through consumption of spices such as tarragon and essential oils derived from spices. The FFHPVC estimates that a worst case daily per capita intake ("eaters only") of estragole from all sources is less than 10 micrograms/kg body weight/day.

The common chemical name for estragole is 4-methoxyallylbenzene, and as a terpene derivative it is closely related in structure to other naturally occurring plant constituents containing the 4-alkoxyallylbenzeze nucleus such as:

| Structurally Related Chemicals | CAS Number |
|---|---|
| Methyl eugenol | ----- |
| Elemicin | ----- |
| Myristicin | ----- |
| Safrole | ----- |

The FFHPVC states that key data on the p-alkoxyallybenzene derivatives (above) provide a more comprehensive chemical, biological and toxicological characterization of estragole.

Finally, another structurally related substance is anethole (CAS No. 104-46-1), differing only in the position in the side-chain double bond. At higher levels of intake (50 – 100 mg/kg bw/day), estragole participates in a metabolic pathway that is associated with hepatic toxicity while anethole participates primarily in a detoxification pathway. Therefore, the FFHPVC only considers those human toxicity data on anethole relevant to estragole toxicity in those studies in which both substances participate in common pathways of metabolic detoxification.

We agree with the FFHPVC on the above conclusions. We also agree with the FFHPVC in their analysis of the available toxicity data and concur that no new studies are needed in rats or mice to meet HPV/SIDS requirements.

At this time, however, we question the FFHPVC's assessment that an acute fish toxicity study (OECD 203) is needed to meet the requirements of the HPV program.

Section 3.3.1 (Acute Toxicity to Fish) of the test plan, as well as the Robust Summaries, provide acute toxicity values in rainbow trout and bluegill sunfish (96-hour $LC_{50}$ for methyl eugenol = 6 mg/L and 8.1 mg/L, respectively). In fathead minnows, using the continuous flow method, the 96-hour $LC_{50}$ = 7.69 mg/L for anethole. Finally, a calculated $LC_{50}$ is given for estragole using ECOSAR EPI Suite 2000 ($LC_{50}$ = 4.56 mg/L).

It is worthy to note that the test plan states "although the data for methyl eugenol, anethole [measured values] and estragole [estimated value using ECOSAR] **consistently show an $LC_{50}$ value of 5 - 10 mg/L** (emphasis added), given the animal toxicity of estragole at high dose level, it is suggested that an $LC_{50}$ be performed for estragole using standard OECD Guideline 203 protocol." We strongly believe that this violates the October 1999 agreement to minimize new animal tests and avoid a box checking exercise. The available data on closely related chemicals and the EPA's model calculation for $LC_{50}$'s are consistent and obviate the need for any new tests on estragole itself in fish. The SAR bridging from actual data on methyl eugenol and anethole to the ECOSAR calculated value for estragole are sufficient to meet this SIDS data requirement. It is not justified to poison 40 fish merely to "refine" the ECOSAR calculation when data are available on structurally related materials and especially when all $LC_{50}$ values are almost identical. This is an illustration of the needless poisoning of fish merely to check a box. Indeed, the FFHPVC also calculated values for melting point MPBPVP EPI Suite 2000), partition coefficient (KNOWWIN EPI Suite 2000), photodegradation (AOPWIN EPI Suite 2000) and fugacity (Level III Fugacity-based Environmental Equilibrium Partitioning through EPA Suite 2000) and considered these requirements fulfilled with no further testing proposed. ECOSAR should be no different in this instance.

If the FFHPVC wishes to study acute fish toxicity, we urge it to use TETRATOX as a means to do so, especially given the fact that this is a GRAS compound and occurs naturally in the

environment. As recently as October 23, 2001, PCRM and PETA coordinated a meeting at EPA to review and facilitate incorporation of an *in vitro* aquatic test into the HPV program. At that meeting, Dr. Terry Schultz, professor of predictive toxicology at the University of Tennessee College of Veterinary Medicine, presented his *in vitro* aquatic toxicity method. Overall, the extensive available information demonstrates TETRATOX is a high quality surrogate for fish testing. In fact, this method is used extensively in private industry and is being considered for regulatory acceptance by OECD.

The details of this meeting and our proposal were detailed in a letter to Stephen Johnson, Assistance Administrator, on December 5, 2001 by PCRM staff scientist Nicole Cardello. To date, after over one full year, there has been no response from Mr. Johnson or anyone else in the agency. A copy of this letter is attached for you reference. **We again respectively request a thoughtful, scientific and specific reply to this letter.** It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this is an ideal opportunity for action over words.

The stated proposal of the FFHPVC to do aquatic testing on estragole presents a perfect opportunity to use TETRATOX. Not only are there existing $LC_{50}$'s on closely related chemicals (to estragole), but there is also an EPA ECOSAR calculated $LC_{50}$ for estragole itself - and all of these $LC_{50}$'s (both test and calculated) are very close to one another (from 5 – 10 mg/L). TETRATOX could be used to further refine estragole aquatic toxicity without poisoning more animals.

I look forward to a prompt and favorable response to our concerns. I may be reached at 202-686-2210, ext. 302, or via email at *csandusky@pcrm.org.*

Sincerely,

Chad B. Sandusky, Ph.D.
Senior Toxicologist

Attachment: Letter, Cardello to Johnson, December 3, 2001

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December 5, 2001

Mr. Stephen Johnson
Assistant Administrator
U.S. Environmental Protection Agency
Ariel Rios Building
Room 3000, #1101-A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Dear Mr. Johnson:

I hope this letter finds you well. I am writing on behalf of the 100,000 members of the Physicians Committee for Responsible Medicine (PCRM) interested in the development and regulatory acceptance of newer, more precise, nonanimal toxicity test methods.

I would like to tell you about an opportunity for the EPA to take a proactive step toward reducing the number of animals killed under its High Production Volume (HPV) Chemical Challenge. As you know, one of the six SIDS endpoints under the HPV program is ecotoxicity, which includes tests on algae, daphnia, and fish. This appears to be an appropriate time to replace the acute fish toxicity test with an established *in vitro* method for predicting aquatic toxicity.

On Tuesday, October 23, 2001, Sherry Sterling, Jessica Sandler of People for the Ethical Treatment of the Animals (PETA), and I coordinated a meeting at the EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program. We invited Terry Schultz, Ph.D., professor of predictive toxicology at the University of Tennessee College of Veterinary Medicine, to present his *in vitro* aquatic toxicity method, TETRATOX. TETRATOX has been standardized and has consistently yielded strong statistical agreement with the *in vivo* aquatic toxicity test. Where results from the two methods diverge, there are reasonable explanations. Overall, the extensive available information demonstrates TETRATOX to be a high quality surrogate for fish testing. In fact, this method is used extensively in private industry and is being considered for regulatory acceptance for a similar application by the Organization of Economic Cooperation and Development.

I was troubled to find that the EPA staff present at the meeting were not particularly interested in our contention that TETRATOX was ready for inclusion into the HPV program, because the attendees mainly deal with pesticide issues. TETRATOX is not

appropriate for pesticides, but it is entirely appropriate for industrial compounds, such as the HPV chemicals.

In a November 30, 2001, telephone conversation with Phil Sayre, Ph.D., associate director of the Office of Pollution Prevention and Toxics' Risk Assessment Division, Dr. Sayre stated that the EPA is not interested in the *in vitro* test because it provided no information on chronic toxicity. However, as you know, chronic fish toxicity is not an endpoint in the HPV program. As with all alternative methods, TETRATOX should be considered for the specific endpoint(s) for which it is appropriate, and not discounted because it cannot cover all possible endpoints. In this case, TETRATOX is an acceptable replacement of the acute fish toxicity screening test required under the HPV program.

In our October 17, 2001, meeting with you, you reaffirmed the EPA's commitment to animal welfare in the HPV program. One of the principles of the October 1999 Agreement reflects the EPA's expectation that nonanimal test methods for some SIDS endpoints may soon be available for replacement of the animal studies. To show good faith in the commitment made in the October 1999 Agreement, the EPA should actively pursue inclusion of nonanimal test methods wherever possible.

PCRM is concerned that large numbers of fish are being killed in the HPV program and other EPA programs, when established *in vitro* aquatic toxicity tests, such as TETRTOX, are available. Furthermore, some of the tests on fish that have been proposed are completely unnecessary given the physicochemical properties of some of the compounds.

- For example, under the HPV program thus far, the American Petroleum Institute proposed acute toxicity tests on fish with volatile substances such as the petroleum gases ethane, butane, isobutene, and propane.

- Three of the Flavor and Fragrance High Production Volume Consortia test plans called for fish toxicity tests with Generally Recognized As Safe chemicals including cinnamyl derivatives, which is essentially cinnamon oil.

- Fatty acids, including oleic, linoleic, stearic, and palmitic acids, have been proposed for fish tests. As you know, oleic acid is the main component of olive oil. Without a detergent or other additive, the olive oil will simply float at the top of the water. Moreover, detergents have toxic properties that make the results of these experiments questionable at best.

- Tests on fish have been proposed with corrosive chemicals that must first be neutralized to bring the pH to a level that will not kill the fish. Companies acknowledge that this changes the fundamental composition of the material and that such test conditions make the results meaningless. Nevertheless, they continue to propose and conduct the tests in order to "satisfy the EPA."

PCRM agrees with PETA that the EPA should take proactive steps in facilitating the incorporation of the TETRATOX assay into the HPV program. We request that the EPA

direct some of the $500,000, which still has not been adequately accounted for, toward the sponsorship of the TETRATOX assay through the Science Advisory Board (SAB) or the Interagency Coordinating Committee on the Validation of Alternative Methods.

Specifically, we would greatly appreciate a response to the following questions:

- What is the extent of the EPA's interest in TETRATOX?
- Is the EPA's interest in using TETRATOX as a surrogate for the acute aquatic toxicity test in the HPV program compromised because it does not address chronic toxicity?
- Is TETRATOX a method that the EPA thinks has inter-agency applicability and therefore should be sponsored through ICCVAM? If so, is the EPA willing to use some of the $500,000 to sponsor the test through ICCVAM? If not, is the EPA interested in reviewing TETRATOX through the SAB/SAP process?

Thank you for your consideration on this important matter. I look forward to your response. I can be reached at 202-686-2210, ext. 302, or at 5100 Wisconsin Ave., N.W., Suite 400, Washington, DC 20016.

Sincerely,

Nicole Cardello, M.H.S.
Staff Scientist